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14. ABSTRACT
The purpose of this contract is to carry out emerging infectious disease surveillance in Kenya. Specific areas in which work is performed include respiratory illness surveillance (particularly influenza), acute febrile illness surveillance, malaria resistance surveillance, diarrhea etiology and antimicrobial resistance surveillance, sexually transmitted illness surveillance, and capacity building. KEMRI maintained surveillance sites in both Kenyan Defense Forces and Ministry of Health clinics and hospitals throughout Kenya. KEMRI operated reference laboratories for this work in Nairobi, Kericho, and Kisumu, including the arbovirus reference laboratory, the antimalarial resistance laboratory, entomology facilities, the Center of Excellence in Microscopy, the microbiology reference laboratory. Capacity development projects include outbreak investigations, Ebola and Marburg virus testing, and continuation of a laboratory and medical maintenance student attachment program. The program was able to serve as the hemorrhagic virus reference laboratory for East Africa, determine etiologies of diarrheal illnesses and the antimicrobial resistance patterns of bacterial causes, determine the etiologies of sexually transmitted infections and acute febrile illnesses in military and civilian populations, and monitor the pattern of antimalarial resistance across Kenya.

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INTRODUCTION:

KEMRI supports USAMRU-K's maintenance of an emerging infectious disease surveillance network by providing contract personnel, laboratory and administrative facilities, capacity development capabilities for contracted personnel and partner organizations, regulatory oversight, and other required functions for the performance of infectious disease surveillance and research. The areas of research/surveillance performed are categorized by the pillars as defined by the US Department of Defense's Armed Forces Health Surveillance Center Division of Global Emerging Infectious Disease Surveillance and Response Operations (DoD-GEIS). These pillars include respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission. This year saw significant changes. Because of funding cuts, many civilian sites were closed with operations transferring to KDF sites. However, because of security concerns, the KDF did not allow our employees onto their bases, so civilian sites were reopened.

BODY: For clarity's sake, this report will be divided by DoD-GEIS pillar.

Respiratory Illness:

Global influenza surveillance to detect viral antigenic drifts and shifts must be reliably undertaken to protect public health. Sub-Saharan African countries have limited laboratories and programs to conduct sustained influenza surveillance. To address this problem, USAMRU-K-GEIS and KEMRI developed a human influenza sentinel surveillance program at 8 civilian hospitals and 2 Kenyan military hospitals since 2006. However, in this fiscal year, of the National Influenza Center and influenza surveillance at civilian sites were handed solely to the Kenyan Government and the US CDC to avoid duplication of effort. Limited surveillance at KDF sites has been maintained under this contract at both Kahawa Barracks in Nairobi and 9KR in Eldoret.

Between October 1st and December 31th 2013, we received 52 NP swab specimens at the central laboratory from ILI patients participating in the regular USAMRU-K sentinel surveillance network. All these were processed and tested for presence of influenza viruses and 10 are pending analysis. In addition, 8 NP swab specimens were received from SARI patients and processed for detection of respiratory viruses during the same period. Influenza viruses In the 4th quarter report of FY13 we reported that 10 NP samples were pending analysis, awaiting viruses testing and possible isolation by inoculation in MDCK cells and that these results would be reported this quarter. PCR results showed that none of the samples had influenza viruses in them. Furthermore, inoculation in appropriate cell lines did not yield any respiratory virus. Thus all these samples were confirmed not to have any of the common respiratory viruses in them. During the 1st quarter of FY14, 3 (6%) of the specimens collected from ILI patients tested positive for influenza by real time RT PCR. Of these, 1 (33%) were influenza A and 2 (67%) were influenza B's. The influenza A case was a female patient aged 40 years whereas the influenza B cases were male patients aged 41 and 54 years respectively. When the single influenza A positive sample was sub-typed using PCR, it was found to be A(H3N2). No seasonal A(H1N1) or influenza A (H1N1)pdm09 subtype viruses were detected. Seasonal A(H1N1) cases were also not identified and were last seen in Kenya in November 2009. Thus, just like the second and third and fourth quarters of FY2012 and the four quarters of FY13, seasonal influenza A (H3N2) was the predominant subtype circulating in Kenya in the first quarter of FY2014. Of the 3 influenza A PCR-positive samples, only a single influenza B-positive sample yielded an isolate upon inoculation in MDCK cells. HAI results showed that the influenza B isolate obtained was B/Wisconsin/1/2010-like (B/Yamagata lineage). The influenza A(H3N2) and influenza B samples that did not yield isolates had high ct values and these were unlikely to yield virus isolates because of the low virus load in the samples. The 2 influenza A(H3N2) PCR-positive samples that were pending inoculation in

MDCK cells and whose results were to be reported in this quarter did not yield virus isolates, again due to low virus loads in the patient samples. Amongst the 8 SARI cases from which NP swabs were obtained, no case tested positive for an influenza virus by PCR. Non-influenza viruses In the first quarter of FY14, 9 non-influenza respiratory viruses were isolated through inoculation of NP samples in appropriate cell lines. All of these were from the ILI surveillance network; the SARI cases did not yield any virus isolate. The samples yielded the following non-influenza isolates: 1 adenovirus, 2 parainfluenzavirus type1, 1 parainfluenzavirus type 2, 4 parainfluenzavirus type 3 and 1 enterovirus. Amongst these, we had one case of dual infection with parainfluenzavirus types 1 & 2. Sequencing work During this quarter, we sequenced 13 amplicons (1.09kb each) of enterovirus serotype 68 Vp1 gene from amongst enteroviruses obtained from our surveillance program. We also sequence 3 full genomes of poliovirus isolates obtained from our respiratory surveillance samples and confirmed that 2 were poliovirus serotype 3 and was 1 poliovirus serotype 1. All the polioviruses isolated were vaccine-like and we are in the process of releasing the data into the public domain. The ability to capture poliovirus infection amongst patients with acute flu-like symptoms in our surveillance network underscore the maturity and early warning capability the program has built at USAMRU-K not only for Kenya but also for region and the world in general. We sized 112 samples from the GEIS-supported MDR program at Kisumu using the fragment analysis facilities on the ABI 3500XL genetic Analyzer. We used 12 neutral microsatellites to determine the structure and how dynamic the P. falciparum parasite in Kenya is. We also sequenced 5 amplicons of a 401 bp fragment of the citrate synthase gen (gltA) for Rickettsia for the AFI program at USAMRU-K and 4 amplicons of a 687 bp fragment of the repetitive transposing-like IS 1111 region for Coxiella burnetii. MUWRP Q1 See attachment MUWRP results GVF See attachment University of Buea LEID Q1 A total of 83 nasopharyngeal swabs were collected and analyzed between October and December 2013 by real-time PCR assay using primers designed by CDC/WHO. All Influenza positive samples are then inoculated in MDCK cells in an attempt to isolate the viruses. Recovered viruses are subtyped by HA/HAI and IFA. Sample collection for influenza surveillance this quarter is lower than in FY12 quarter 1 and FY13Q1 during which 149 and 152 samples respectively were collected. We did not receive any case for outbreak investigation; all cases were collected for regular sentinel surveillance. A total of eight (08) cases of SARI were detected. Among these, one sample was infected with influenza B virus. Out of the 83 samples analyzed by real-time PCR, influenza virus (Flu A or Flu B) was detected in 12 (14.46%). We identified Influenza B in 03 (25%) of the 12 influenza positive samples and influenza A in 09 (75%) of the positive samples. Of the Flu A positive samples were infected with A(H3). Contrary to Q1 of FY12 and FY13 where flu B dominated, we saw a dominance of A(H3) this quarter. However, similar to Q1 of FY13, we did not detect the pandemic strain A(pH1H1) this quarter. The circulation of flu viruses (14.46%) was similar to what we obtained in Q1 of FY13 (14.47%) but lower than FY12 Q1 (24.16%) and FY 11 Q1 (18.5%). This confirms our earlier assertion that as we progress away from the H1N1 pandemic, we have noticed a decline in influenza activity suggesting that the high prevalence recorded in the first quarter of FY11 and FY12 may have been influenced by the circulation of the 2009 pandemic strain. Viral recovery is still in process as we had to optimize our methods as we try to use cheaper alternatives in the tissue culture facility. PAI Q1 A total of 199 patients were enrolled between October and December 2013, samples were tested against a number of respiratory viruses using singleplex real time reverse transcription polymerase chain reaction (RT-PCR). Results indicate 12 (6%) patient tested positive for Flu A subtype H3 and four (2%) patient were positive for Flu B.

January to March 2014, 375 samples were collected and screened by PCR for flu A with an overall prevalence of 3.5% (Table 3) and a prevalence of 1.9% for the A(H1N1)pdm09 strain (Table 4). The overall prevalence of flu B positives was 1.1% during this quarter (Table 5). The results indicate an increase in Flu A positivity and a significant decline in Flu B positivity in comparison to the previous period (Tables 3 and 5). Summary of the sub-types

indicate that A(H1N1)pdm09 and H3 continue to co-exist though A(H1N1)pdm09 was the predominant subtype during this quarter (Table 6). MUWRP established a working relationship with CEIR of University of Minnesota as mechanisms for sharing samples and maintaining an EQA/QC collaboration for influenza testing Human Component. During the 2nd quarter of FY14, 8 (11.3%) of the specimens collected from ILI patients tested positive for influenza by real time RT PCR. Of these, 7 (87.5%) were influenza A and 1 (12.5%) was influenza B. The influenza B case was from a 9-year old male patient. Amongst the patients infected with influenza A viruses six cases (85.7%) were aged =5 years and a single case (14.3%) was from a patient who was older than 5 years. When the six influenza A positive samples were subtyped using PCR, it was found that five were A(H3N2) and one was of A (H1N1)pdm09 subtype. Thus unlike the first quarter when only A(H3N2) viruses were detected in the second quarter influenza A (H1N1)pdm09 subtype virus was detected. Seasonal A(H1N1) cases were not detected and were last seen in Kenya in November 2009. Thus, just like the second and third and fourth quarters of FY2012 and the four quarters of FY13, seasonal influenza A (H3N2) was the predominant subtype circulating in Kenya in the second quarter of FY2014.

April 1 - June 30, 2014, Metabiota provided essential surveillance support was to the Ministry of Health and Centre Pasteur (NIC) through the identification and testing of patients with Influenza-Like Illnesses (ILI). During this time, we collected a total of 63 samples. Sixty-two of these were screened during the quarter. The last remaining sample will be tested during the first week of July. Among those screened, seventeen samples tested positive for flu (27.4%). Eleven samples subtyped as A/H3N2 (17.74%) and six samples subtyped as Influenza B (9, 67%). Kenya: Between April 1st and June 30th 2014, we received 25 NP swab specimens at the central laboratory from patients participating in the regular USAMRU-K sentinel surveillance network. All these patients had ILI and none was a SARI patient. All specimens received at the lab were processed and tested for presence of influenza viruses. Tanzania: As noted in the results table below , the influenza surveillance activities in all the three sites have continued smoothly, the sites have recruited a steady number of patients into the study. The program has maintained good weekly reporting system to all GEIS collaborators as well as sharing aggregate data with the Ministry of health for monthly reporting to WHO. Uganda:MUWRP continues through its collaboration with Makerere University College of Veterinary medicine, and other institutions to provide capacity building and training to students (undergraduates and graduates) and partner staff through samples collection and lab diagnostics as a means of building national virologists capable of responding to pandemic threats. MUWRP previously trained a laboratory technologist from the National Animal Disease Diagnostic and Epidemiology Centre (NADDEC) a branch of the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) in Sample Accessioning and Archiving. During this quarter two students doing biomedical laboratory technology are doing their internship. During this quarter MUWRP developed and installed a Sample Label's Laboratory System for the National Influenza Centre and provided training to the lab staff. The sample label system was initially supported by CDC. During this quarter, MUWRP hosted the MUWRP-UVRI Management meeting. The meeting between MUWRP and the Uganda Virus Research Institute/National Influenza Center are held twice annually to enhance the collaboration between the two institutions. The meeting provides updates on the various activities and possible areas of further collaboration. Also discussed during the meeting was the need to review and renew the MOU to include other areas of interest. The meetings are chaired by the MUWRP Executive Chair on MUWRP side and by the Director of UVRI. Among those screened, seventeen samples tested positive for flu (27.4%). Eleven samples subtyped as A/H3N2 (17.74%) and six samples subtyped as Influenza B (9, 67%).See attached report for graphs. Cameroon, LEID: See attached Kenya: In the 2nd quarter report of FY14 we reported that we reported that 5 A(H3N2), 2 A (H1N1)pdm09 and 1 Flu B samples positive by real-time RT-PCR were pending to be worked on in the 3rd quarter. All these samples were inoculated into cultured MDCK cells and only the Flu B positive sample yielded a 2013 southern hemisphere vaccine strain

B/Wisconsin/1/2010-like virus. All, the influenza A samples did not yield a virus isolated under MDCK cell line. During the 3rd quarter of FY14, 3 (12%) of the specimens collected from ILI patients tested positive for influenza by real time RT PCR. All the three (100 %) were influenza A and there was no influenza B detected. When the three influenza A positive samples were sub-typed using PCR, it was found that one was A(H3N2) and two were of A (H1N1)pdm09 subtype. The A(H3N2) virus was isolated from a 26 year-old male patient from Mtongwe naval base whereas the first of the A (H1N1)pdm09 virus was from a 27 year-old male patient from Kahawa barracks and the other from a 5 year-old female patient from the same barracks. All the cases were detected in the month of April. Thus, A(H3N2) and A (H1N1)pdm09 viruses co-circulated in the third quarter in Kenya. Seasonal A(H1N1) cases were not detected and were last seen in Kenya in November 2009. We detected 2 cases of infection with a non-influenza respiratory virus after inoculating the NP samples in appropriate cell lines. All of these were from the ILI surveillance network. The two cases yielded a pan-enterovirus each. Both cases were male patients; the first was 3 years11moths from Mtongwe naval base and the second was 3years and 6 months from Kahawa baracks.During this quarter, the the ABI 3500XL genetic Analyzer was upgraded to make it cost effective for sequencing using reagents that may have expired but which had not been opened. Due to expiration of the WRAIR#1267 protocol, during this quarter we focused on sequencing virus isolated obtained from the approved students' projects. 1. For the MDR team from Kisumu, 66 samples were analyzed for fragment analysis for 8 loci. Thus the total number of reactions carried out was 528. Prior to expiration of the protocol we sequenced the 15 full-length NA fragments of influenza B isolates. We also sequence 44 influenza A isolates for the targeting full length HA, NA and M segments. Furthermore, 15 500bp PCR amplicons of human Rhinovirus isolates were sequenced as were 3 PCR amplicons of a 550bp amplicon of Corona Virus RdP gene. Finally, one PCR amplicon of a Chikungunya virus gene from the VHF group was sequenced. These sequence data are undergoing analyses Tanzania.

Between July 1st and September 30th 2013, no work was performed due to loss of funding and expiration of protocol.

Acute Febrile Illness:

The project continued to play a key role in surveillance work for pathogens associated with febrile illnesses, outbreak response and training of MOH personnel. The USAMRU-K FVBI program conducts surveillance for pathogens associated with acute febrile illness through coordinated human case detection and vector surveillance. This way, the program is able to better characterize public health and community risk of FVBI in different parts of Kenya and thereafter provide report surveillance data to clinical sites, MoH and Veterinary Department (for zoonoses). This program has two components: the arbovirology that conducts field and diagnostic testing of entomologic and human specimens of all suspect VHF. The program also tests for VHF in samples collected through the Acute Febrile Illness protocol, and regional WHO reference specimens for arboviruses. Acute Febrile Illness: This project conducts spatial and temporal pathogen surveillance to identify the causes of undifferentiated febrile cases through a multi-site testing algorithm. Horn of Africa Leishmania Surveillance: This project surveys for leishmania infected sandflies in the region.

The entomology group carried out numerous activities. In Q1 sand flies collected from Marigat, Lodwar, and Gilgil were stored in cryovials containing 70 % ethanol, labeled according to site, date of collection and trap number, and later shipped to Kisian entomology laboratory for processing. Collected samples were then dissected, cleared, mounted and identified to species. A total of 440 male and female sand flies were identified. The abdomens from the female flies were preserved in -80degrees C to be tested for pathogen infection as soon as the testing reagents become available. One mosquito surveillance trip was completed within this quarter from 4-21 December 2013. Samples were collected from Marigat district within

Baringo County at four sites (Logumgum (sampled twice); Longwan; Salabani (sampled twice); and Sirata) that had served as foci for the last Rift Valley fever virus (RVFV) outbreak. Increased precipitation and changes in vegetation based on NASA data within this period (November and December) raised concerns of possible RVF outbreak. Collected samples have been stored at -80 degrees C and will later be sorted, identified to species, and tested by RDT and PCR for RVFV infections. The fleas trapped during previous field collections were identified to species and genus where applicable. These were pooled and total nucleic acid (RNA and DNA) extracted. A total of 76 pools were realized. 440 species of sand flies identified to species, 76 pools of rodent fleas tested with 8 pools being positive for rickettsiae infection, and over 450,000 mosquitoes collected for RVF virus testing. In Q3, approximately 2300 adult sand flies collected, and about 230 identified. In Q4 several vectors (Mosquitoes and sandflies) were collected across different sites during this quarter. Approximately 2800 sand-flies were collected from 3 sites in Merti and brought back to the USAMRU-K Entomology labs for identification. Mosquitoes (~6500) were also collected in Budalangi site and have been identified and pooled for testing for arboviruses. Approximately 7,003 mosquitoes were collected in Marigat as part of the pre-flooding Rift Valley fever (RVF) surveillance. Also 55 mosquito larval habitats were mapped in Kisii County in readiness to conduct a trial on larval control using BTI.

The arbovirology/VHF unit remained active. In Q1, a team of four members from the entomology lab travelled to Mombasa, KDF 17 KR barracks at Nyali between November 28th and December 2nd following laboratory confirmation of dengue cases. The objective was to assess the entomologic risk for transmission of dengue fever and come up with recommendations to be shared with K-DOD in order to mitigate against the problem. 1321 mosquitoes were collected. Additionally, 37 human samples were received and tested for dengue virus. In Q2, approximately 207,000 mosquitoes have been identified and pooled into 266 pools, 1000 adult sandflies collected for P. orientalis colony propagation, mites identified, pooled and tested for Rickettsiae and Scrub typhus. In addition, 440 species of sand flies identified to species, 76 pools of rodent fleas tested with 8 pools being positive for rickettsiae infection, and over 450,000 mosquitoes collected for RVF virus testing. Also 119 human samples were received and tested. In Q3 two field trips were conducted to collect vectors for routine arbovirus surveillance. Between May 27th and 31st, a team of five members from the entomology lab sampled mosquitoes in Sukari ranch in Ruiru, Kiambu County. A total of 10,070 mosquitoes were collected, pooled, identified and pooled into 379 pools. Between June 22nd and 29th, another team of 5 members travelled to Entasopia, Magadi County, in the Rift Valley for the same exercise. Over 600 mosquitoes were collected, identified and pooled into 69 pools. Also approximately 1,200 ticks were collected form livestock and have been lined up for identification in the coming weeks. Lab: Forty two Human samples from the Mombasa dengue outbreak were inoculated in Vero cells.Similarly ,194 mosquito samples collected during the dengue outbreak were also tested.All probable isolates obtained in this quarter were re-inoculated to confirm reproducibility and those positive were forwarded for molecular analysis. From April to June 2014, the serology section processed a total of 69 clinical samples from 10 health facilities. The samples were tested for the presence of IgM antibodies for various arboviruses and viral hemorrhagic fever agents. 1 sample from Nairobi hospital tested positive for Dengue IgM antibodies. In addition, the laboratory also ruled out rift valley fever from 10 samples received from Muranga County who had asked specifically for rift valley fever testing on the samples. In Q4 field entomologic sampling was performed from 17th to 24th July 2014 in Baringo county-Rift Valley Regionntry. Samples collected include mosquitoes, sandflies and ticks. A total of 22,454 mosquitoes were collected, identified and pooled into 1,169 pools. From July to September 2014, the serology section processed a total of 73 clinical samples from 19 health facilities in Kenya and regionally (Somalia and South Sudan). The samples were tested for the presence of IgM antibodies for various arboviruses and viral hemorrhagic fever agents. Three samples (2 from Malindi and one from Ngararia health

center) tested positive for IgM antibodies against Rift Valley fever and results relayed to the ministry of health. Four samples (2 from the defencse memorial hospital, 1 from langata health center in Nairobi and 1 from malindi) tested positive for IgM antibodies against Dengue fever .

In the BSL laboratory in Q1, the project continued to play a key role in pathogen surveillance and disease outbreak response. In Q2, a cumulative total of 381 samples were collected in the reporting quarter, and 200 tested for the following pathogens: malaria, tick borne zoonoses (rickettsiae, Q-fever, Bartonella, Borrelia, Anaplasma, Ehrlichia and Babesia), Dengue, leishmania, leptospirosis, brucellosis, Salmonella and EBV. Unusual reports at each surveillance sites discussed with relevant health officials. The Laboratory assisted in outbreak investigation for a disease that had killed 6 individuals in Baringo County. Blood samples were taken from families and neighbors of cases, including 25 adults and 6 children. No significant travel or antecedent medical history was elicited from these cases, however, contact with cows, goats, sheep, chicken and dogs and an antecedent history of ill goats in the area was provided. Malaria rapid tests conducted at the sites were negative, however, 6 (19.4%) and 12 (38.7%) of 31 samples were found to be highly positive by quantitative polymerase chain reaction (qPCR) and anti-phase 2 antibody, for Q fever (i.e, diagnostic for C. burnetii infection). Additionally, 5 samples were found to be positive for Salmonella spp. by qPCR (potentially reflective of typhoid fever). Further testing for arbo virus (dengue, RVF, yellow fever and Chikunguya), rickettsiae, B. burgdorferi, anaplasmosis, ehrlichiosis, leptospirosis, brucellosis, tick-borne relapsing fever and bartonellosis are negative. Considering that Q-fever and salmonella are, endemic in the region, we have sought permission to send samples to CDC Atlanta and WRAIR (Division of Virology) for further testing with unbiased diagnostics that are currently unavailable in the Lab. In Q3 a cumulative total of 295 samples were collected in the reporting quarter, and 223 tested for the following pathogens: malaria, tick borne zoonoses (rickettsiae, Q-fever, Bartonella, Borrelia, Anaplasma, Ehrlichia and Babesia), Dengue, leishmania, leptospirosis, brucellosis, Salmonella and EBV. In Q4 a cumulative total of 276 samples were collected in the reporting quarter, and 96 tested for the following pathogens: malaria, tick borne zoonoses (rickettsiae, Q-fever, Bartonella, Borrelia, Anaplasma, Ehrlichia and Babesia), Dengue, leishmania, leptospirosis, brucellosis, Salmonella and EBV. In addition to the 228 samples from camel keeping regions that were tested for MERS-ConV (see QTR3 report), an additional 872 were tested in the reporting period. Of the cumulative total of 1100, 18 (~2%) have titers considered positive for MERS-ConV. MERS-ConV reactors will need to be confirmed by neutralization assays. Prof Drostein team from from Bonn University, Germany will travel to Kenya and provide reagents and protocols for neutralization assays in a BSL 3 lab at KEMRI. If confirmed, this could be the first demonstration of MERS-ConV infections outside the Arabian Peninsula.

Malaria:

The Malaria Drug Resistance laboratory performs both in-vivo and molecular analysis to track antimalarial resistance patterns in the region. USAMRU-K, Malaria Diagnostics Centre (MDC) supports WRAIR's clinical trials on malaria vaccines, drugs, and other compounds. The MDC also conducts malaria diagnostic training using standardized skill-based training and assessment programs in a bid to improve competency and strengthen microscopy diagnosis. Other activities include preparation of reference malaria blood films and characterization of specimens for evaluation and optimization of novel malaria diagnostic methods. Methods: (1) Sustain laboratory capacity with training and proficiency testing for Kenya MOH personnel performing microscopy and RDT; (2) Identify and collect target Plasmodium species; (3) Prepare malaria reference blood films and samples for training, proficiency testing, and for repository for evaluation of alternative malaria diagnostics; (4) Sustain technical competencies

of MDC staff in key areas. Benefits to DOD and science: Provide technical support to WRAIR's and pharmaceutical firms' clinical trials on malaria vaccines, other drugs, and compounds. Improve microscopic diagnosis of malaria for both clinical research and patient care utilization. Maintain a repository of whole blood samples for evaluation of new technologies for malaria diagnosis.

MDL: 1) Published 10 manuscripts including 4 with collaborators. One manuscript published in Science. 2) Additional two manuscripts have been accepted for publication with minor changes requested. 3) One manuscript has been submitted for publication and 5 are in preparation. 4) Five abstracted were submitted to ASTMH 2014. All were accepted. Two won travel awards. 5) We have won a total of 4 travel grants this quarter. 6) Established one new MTA, in the process of establishing 3 new CRADAs. 7) Received and successfully revived artemisinin resistance clonal line that was recently published. 8) Invited to present at 3 important international conferences held in Kenya. 9) Have trained extensively and improved on our quality control/assurance, management and tracking of samples, samples inventory update and control etc. Extensive systems and protocols have been put in place in order to manage the extensive and large inventory of samples in MDR archives/freezers. 10) Two students successfully defended their Masters thesis and two completed their thesis work, waiting to defend. 11) Wrote and submitted 3 protocols for approvals; two of them are nearing final approval. 12) Received and installed Global Biosurveillance Technology Initiative equipments. In Q1 collected 182 clinical samples, performed immediate ex vivo analysis on 107 samples (64 from Kisumu district hospital and 43 from Kombewa district hospital) of which 44 samples were successfully analyzed. DNA was extracted from 182 field samples. Culture adapted and did in vitro drug analysis of 15 historical samples. Performed molecular analysis of ~550 archived historical samples. Analysis was only done on ~5 markers. The target is to analyze ~10-30 markers depending on which samples being analyzed. Supported in vivo efficacy study (Dr. Ben Andagalu study). Performed ex vivo analysis of 66 samples of which 44 were successful. All samples collected from the study were cryopreserved. Performed molecular analysis on 60 samples from the in vivo efficacy study. In Q2, In vitro drug sensitivity: 345 P. falciparum (Pf) specimens were collected from Kisumu (130), Kombewa (101), Kisii (85) Marigat (8) and Kericho (21). Additionally, 36 Isolates from in vivo study. Subsets of sample isolates from Kisumu and Kombewa district hospitals were analyzed for immediate ex vivo and the rest cryopreserved. Samples from Kisii, Kericho and Marigat were cryopreserved for future culture-adaptation and in vitro sensitivity testing. 35 assays were run in quadruplets for select isolates using the standard MDR drug panel. Findings are summarized in table 1. For the artemisinin derivatives specific assay, an additional 25 assays were performed for 8 drugs, in quadruplets to give 100 tests for each drug. Molecular analysis: Through collaboration with IHI in Tanzania, Sequenom MassArray was used to analyze MDR SNPs. We assayed a total of 27 SNPs including PfMDR1 (86,184,1034,1042,1246), PfCRT (72,76,271,326,356,371), PfDHFR (16,22,59,108,164), PfDHPS (463,437,581,613), PfCYTB (133,268,284), PfMRP1 (191,437,876,1390) for a total 384 archived samples. For genome wide analysis, SNP were assayed on 269 samples (32,811 reactions) collected 2000-2003, and 115 in vivo samples (13,915 reactions). Sequencing of the PfMDR 1 gene using ABI3500xL was performed to determine SNPs conferring MDR on 300 samples (600 reactions). In addition, 298 samples (3576 reactions) were assayed for 12 Microsatellites located across the Plasmodium genome to determine the genetic diversity and population structure of parasites in Kenya by fragment analysis on state-of the-art 3500xL. In the 3rd quarter of FY14, 284 P. falciparum specimens were collected from 5 sites namely: Kisumu (84), Kombewa (91), Kisii (84) Marigat (10) and Kericho (12). Additionally, 3 Isolates form the ongoing in vivo study were received. Subsets of these samples, from Kisumu and Kombewa district hospitals were analyzed for in immediate ex vivo and the rest cryopreserved. Samples from Kisii, Kericho and Marigat were culture-adapted and assayed for in vitro susceptibility then cryopreserved for future reference. All drug testing assays were conducted alongside

Culture-adapted index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. The panel of antimalarial drugs screened were mainly artemisinin based derivatives and their partner drugs including dihydroartemisinin (DHA), amodiaquine (AQ), lumefantrine (LU), Mefloquine (MQ), Piperaquine (PPQ), artemether (AT), artemisinin (AR), artesunate (AS). Expanded panel further included other antimalarials of public health interest such as arteether (AE), atovaquone (AV), chloroquine (CQ), doxycycline (DX), halofantrine (HAL), primaquine (PQ), quinine (QN), tafenoquine (TQ). For the whole panel of 16 antimalarials, a total of 40 assays were run in quadruplets for selected isolates to give 160 assays for each drug. Findings are summarized in table 1 of attached report by PI. MDC:88 volunteers have been screened for malaria and positive samples collected for blood films, whole blood aliquots and blood spots preparation. The blood films have been used to conduct I QA/QC training for 20 personnel from the Ministry of Health and proficiency testing for 31 personnel from the Ministry of Health. Molecular analysis: For the third quarter sequencing of the PfMDR1, PfCRT genes using ABI 3500xL was performed to genotype for SNPs conferring resistance to a panel of antimalarials on 228 archival samples (1368 reactions). Data from these assays is still under analysis. Assaying of samples collected in the third quarter is also underway. Please note minimum molecular analysis was performed on samples collected this quarter. We are going to analyze the samples using the Sequenom platform in Tanzania which is more economical and comprehensive compared to Sequencing. However, because we have to travel to Tanzania which involves approval processes and order of reagents, the trip will take place in August-September. During this trip, we expect that more than 1500 samples will be processed in more than 30 molecular markers associated with drug resistance. In the 4th quarter of FY14, 138 P. falciparum (Pf) specimens were collected from 5 sites namely: Kisumu (26), Kombewa (47), Kisii (26) Marigat (28) and Kericho (13). Subsets of sample isolates collected from Kombewa district hospitals were analyzed for immediate ex vivo and the rest cryopreserved. Samples from Kisii, Kericho and Marigat were culture-adapted and in vitro sensitivity testing done. All drug testing were conducted alongside culture-adapted index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. The standard drug panel of antimalarials was tested. A total of 5 samples were run in quadruplets for selected isolates to give 40 assays for each drug. Molecular analysis: Single Nucleotide Polymorphisms (SNPs) associated with drug resistance were analyzed on the Sequenom MassArray platform, through collaboration with Ifakara Health Institute in Tanzania. A total of 1320 samples collected in 2013 and 2014 were analyzed including 120 from the on-going in vivo efficacy study. An attempted was made to assay a total of 27 SNPs including in PfMDR1, PfCRT, PfDHFR, PfDHPS, PfCYTB and PfMRP1 genes for a total of 53,515 reactions. In addition, genome wide analysis of 301 SNPs across the Plasmodium region for a total of 192 (57,792 reactions) samples collected from in 2014. The initial experiments were done but had to be postponed due to mal-functioning of the MassArray. The equipment has been fixed and we are scheduled to return to Tanzania to complete the work before the end of OCT. Sequencing of 756 archived samples (1512 reactions) to determine SNPs within the PfMDR1 gene, 564 archived samples (1128 reactions) to determine SNPs within the PfCRT at 72-76 haplotype, and 706 archived samples (1412) to determine SNPs within the PfDHFR and PfDHPS that confer resistance to a panel of antimalarials were also performed using 3500xL and the 3130xL. Additionally, 252 samples (5292 reactions) were assayed for 21 Microsatellites flanking the pfcrt (8 loci) and pfmdr1 (13 Loci) genes to determine the selective sweeps, genetic lineages and hitchhicking of parasites in Kenya by fragment analysis on state-of the-art 3500xL.

MDC: 494 volunteers have been screened for malaria and positive samples collected for blood films, whole blood aliquots and blood spots preparation. The blood films have been used to conduct the following trainings:- • Basic malaria microscopy: 29 personnel from the Ministry of Health,12 from East African Malaria Task Force, 3 from KEMRI/CDC, 1 from WRP-Makerere university, I from Blantyre Malaria Project in Malawi, 2 personnel from the KDF, 2 from KEMRI-Nairobi, 4 from WRP-Kisumu and 2 from WRP-Kericho. • NICD malaria

microscopy proficiency certification of 8 MDC, laboratory technicians and 2 KDF military personnel. All were scored as experts. • Train the trainer (ToT) level I certification course for 16 MDC, 11 entomology personnel and 4 KDF officers. • WHO microscopy proficiency certification for 7 MDC personnel and 1 KDF personnel will be conducted by the AFRO Center (AMREF) using current funds on • QA/QC training for 80 personnel from the Ministry of Health • Proficiency testing for 71 personnel from the Ministry of Health, 2 KDF personnel and 1 from KEMRI- Nairobi. In Q2, 151 volunteers have been screened for malaria and positive samples collected for blood films, whole blood aliquots and blood spots preparation. The blood films have been used to conduct 1 basic microscopy course for 31 personnel and proficiency testing for 28 personnel from the Ministry of Health. In Q3 88 volunteers were screened for malaria and 27 tested positive. From these, approximately 1908 blood films, 45 blood aliquots and 15 blood spots were prepared. In Q4 494 volunteers were screened for malaria and 28 tested positive. From these, approximately 7877 blood films, 423 blood aliquots and 28 blood spots were prepared. A total of 221 MoH and KDF personnel were trained.

Enterics:

Acute gastroenteritis is a debilitating disease and is considered a major disease non-battle injury for deployed U.S. military personnel. A clinical surveillance protocol (WRAIR #1549) to identify microbial pathogens from human stool specimens collected at sites within the GEIS network in Kenya is currently being conducted at the Microbiology Hub Kericho (MHK), USAMRU-Kenya. This protocol is a case (volunteers with acute diarrhea) control (age-matched asymptomatic volunteers) study that allows for the collection of stool specimens recruited at an outpatient clinical setting. Briefly, stool specimens are collected in preservation media at the surveillance sites and transported to the MHK where they are processed and tested for bacterial, parasitic, and viral pathogens. Enteric bacteria identification and antibiotic susceptibility are conducted. Ova and cysts of parasites are identified by general and immunofluorescence microscopy. Enteric viruses are diagnosed using either an enzyme immunoassay or with a commercially available multiplex PCR kit for adeno, astro, rota, and norovirus. The greatest benefit to the DoD is having a highly competent microbial disease clinical laboratory that will provide much needed support to the AFRICOM mission. Scientifically, the enterics surveillance conducted is providing valuable data on the prevalence of enteric pathogens in Kenya as well as potential patterns of antibiotic resistance among bacterial isolates in Kenya.

A total of 261 stool samples (128 cases and 133 controls) were received and processed for enteric bacterial, parasitic and viral pathogens during the 1st quarter FY14 from 6 surveillance sites. Pathogen etiologies and antibiotic susceptibility data is recorded in the attached spreadsheet. Port Reitz, Mbagathi, Isiolo District Hospital, New Nyanza, and the Unilever Tea Plantation sites were closed during or shortly before Q1 FY14. Training to KDF personnel in Moi Barracks was administered to 8 KDF clinical officers. The MHK maintains College of American Pathologists (CAP) certification.

A total of 117 stool samples from 73 adults (over 5) and 44 children (under 5) / 63 cases and 54 controls were received and processed for enteric bacterial, parasitic and viral pathogens during the 2nd quarter FY14 from 6 surveillance sites. Pathogen etiologies and antibiotic susceptibility data is recorded in the attached spreadsheets. The current surveillance sites are the Kericho, Kisii, Kisumu, Kombewa District Hospitals and the KDF's Moi Barracks medical clinics, 9KR and RTS, in Eldoret. During this quarter the MHK accepted a number of clinical samples from the Armed Forces Memorial Hospital (AFMH) in Nairobi and Kericho District Hospital (KDH). In March, the MHK lab responded to a major local spike in infant mortality rate at KDH's Newborne Unit (NBU) in which 18 neonates died of unknown causes during a three week period. By providing critical information back to the hospital's administrators on the antibiotic susceptibilities for each environmental and clinical isolate, the hospital – which currently lacks an infection control program – was able to halt the outbreak

and adjust their antibiotic regiment based on the high levels of antibiotic resistance in recovered pathogens. The MHK also provided IDs and ASTs for a burn patient with a resistant Acinetobacter baumannii infection and a young male with a non-healing ulcer on his foot (both in AFMH, Nairobi). The MHK arranged for improvements to be made at Kisumu District Hospital's waiting/reception area that have greatly benefited the site for all GEIS studies currently enrolling subjects in Kisumu DH.

A total of 260 stool samples from 142 adults (over 5) and 118 children (under 5) / 135 cases and 125 controls were received and processed for enteric bacterial, parasitic and viral pathogens during the 3rd quarter FY14 from 6 surveillance sites. Pathogen etiologies and antibiotic susceptibility data is recorded in the attached spreadsheets. The current surveillance sites are the Kericho, Kisii, Kisumu, Kombewa District Hospitals and the KDF's Moi Barracks medical clinics, 9KR and RTS, in Eldoret. During this quarter the MHK continued to receive both clinical and environmental samples from the Armed Forces Memorial Hospital (AFMH) in Nairobi. In May, the AFMH sent several swabs from the hospital's ICU, which the MHK processed and reported back ID and AST information to the hospital. A CSF sample was also referred to the MHK from AFMH; the isolate was identified as an MDR strain of Acinetobacter baumannii infection. Subsequent to the report, the MHK was asked to provide AFMH with continuing medical education (CME) focusing on this isolate. In June, the MHK was inspected and reaccredited by the College of American Pathologists (CAP) certification.

A total of 240 stool samples from 163 adults (over 5) and 77 children (under 5) / 122 cases and 118 controls were received and processed for enteric bacterial, parasitic and viral pathogens during the 4th quarter FY14 from 6 surveillance sites. Pathogen etiologies and antibiotic susceptibility data is recorded in the attached spreadsheets. The current surveillance sites are the Kericho, Kisii, Kisumu, Kombewa District Hospitals and the KDF's Moi Barracks medical clinics, 9KR and RTS, in Eldoret. During Q4 FY14, the MHK completed 100% face-to-face training on biosafety/biosecurity from the WRAIR Biosafety Officer. The MHK also built capacity by training on the MiSeq Next Generation Sequencer as well as the Luminex. Data from the MHK's studies with the British Army was presented at the Royal Society for Tropical Medicine and Health (RSTMH) in September. An agreement to begin a collaborative effort with the CDC for surveillance of rotavirus in children in western Kenya (under MHK's EPS protocol) was approved.

Sexually Transmitted Infections:

Kenya, one of the more prosperous countries in East Africa, patients presenting to Ministry of Heath clinics with complaints suggestive of STIs (discharge or genital ulcer) often go undiagnosed, and are treated empirically with broad spectrum antibiotics. The drug resistance profiles, especially of gonorrhea, is largely unknown. Methods: In partnership with MOH and the KDF, all patients presenting to Kisumu and the Mbagathi District Hospital, the Mtongwe Naval base, Lanet Military barracks clinic and Kahawa barracks clinic with symptoms suggestive of gonorrhea discharge are offered anonymous screening for gonorrhea and chlamydia (GC) and specimen taken for detection and isolation of Neisseria gonorrhoeae. Treatment is provided as per the ministry of Public Health and Sanitation guidelines. Antimicrobial susceptibility was determined using the E test method.

In Q1, Number of individuals screened 246 Number Eligible 199 Number of specimen(genital Swabs) collected: to confirm from Database:199 Number of confirmed N. gonorrhoeae Isolates:38 Number of isolates tested for antimicrobial susceptibility by E-test against all 9 antimicrobial agents in our test panel:35. Number Tested for Chlamydia and N. gonorrhoeae(GC) by Aptima Combo_II Assay(NB: testing started with specimencollected from October 2012 onwards) :83 Number of Specimen Positive for GC by Aptima Combo: :34 Number Positive for Chlamydia:4 : Number Positive for both Chlamydia and GC: 3.

In Q2 Number of isolates tested for antimicrobial susceptibility by E-test against all 9 antimicrobial agents in our test panel: 38 Number of isolates yet to be tested for antimicrobial

susceptibility by E-test: 30 received from Ganjoni Women's Clinic in March 2014. Number Tested for Chlamydia and N. gonorrhoeae(GC) by Aptima Combo_II Assay: 128 Number of Specimen Positive for GC by Aptima Combo:II test 47 Number Positive for Chlamydia::8 Number Positive for both Chlamydia and GC::5.

In Q3 Number of individuals screened: 246 Number Eligible: 232 Number of specimen(genital Swabs) collected: 232 Number of confirmed N. gonorrhoeae Isolates: 50 Number of isolates tested for antimicrobial susceptibility by E-test against all 9 antimicrobial agents in our test panel: 50 Number of isolates yet to be tested for antimicrobial susceptibility by E-test: None. Number Tested for Chlamydia and N. gonorrhoeae(GC) by Aptima Combo_II Assay: 128 Number of Specimen Positive for GC by Aptima Combo:II test 47 Number Positive for Chlamydia::8 Number Positive for both Chlamydia and GC::5.

In Q4 Number of individuals screened: 246 Number Eligible: 232 Number of specimen(genital Swabs) collected: 248 Number of confirmed N. gonorrhoeae Isolates: 58 Number of isolates tested for antimicrobial susceptibility by E-test against all 9 antimicrobial agents in our test panel: 58 Number of isolates yet to be tested for antimicrobial susceptibility by E-test: None Number of specimen collected/tested: 262(inclusive of 30 Isolates received from ganjoni women's clinic in the month of April, 2014). Number Tested for Chlamydia and N. gonorrhoeae(GC) by Aptima Combo_II Assay: 147 Number of Specimen Positive for GC by Aptima Combo:II test 54 Number Positive for Chlamydia:11 Number Positive for both Chlamydia and GC:7.

Capacity Development:

This project supports all unforeseen contingencies such as disease outbreak investigations and diagnostic support of the local public health community. More recently this fund has been used to support outbreak investigation in the cost region of Kenya upon the request of the KDF. The fund is also used to send GEIS personnel to do Safety and QA/QC inspections at the various surveillance sites in the country. It is also the only fund available to send scientists to present at scientific conferences if and when approval is obtainedl. Since we have been told by WRAIR that 29% overhead cost will be applied to all GEIS funding, USAMRU-K GEIS has lost 1/3 of its total budget allocation in FY14. Therefore this funding will have to absorbe more laboratory associated costs this fiscal year than in previous years.

In Q2: On March 11, USAMRU-K received a call from Kijabe Hospital (Kiambu County) due an increase cases of admissions of young children with respiratory disease which was not responding to antibiotics. Within a short time more than ten of these children were admitted, and four had died, one was in ICU and at the verge of death, one in HDU and at least seven in the general wards in various states of severity with respiratory disease/pneumonia. Samples from the patients were collected in VTM and packaged according to GLP and biosafety principles. We collected specimens from a total of seven patients comprising one ICU patient , one HDU patient and the rest from the general ward. At the lab real-time PCR assay was used to analyze for the following respiratory viruses: 1. influenza A (subtypes: seasonal H1N1, pandemic strain, H3N2, H5N1) 2. Influenza B (Victoria and Yamagata lineages) 3. human adenoviruses 4. RSV types A and B 5. Parainfluenzavirus types 1, 2, 3 6. Human Rhinoviruses 7. Non-polio panenteroviruses including Coxsackie types A & B, echoviruses, etc. Two patients had respiratory viruses. One of the patients was positive for parainfluenzavirus type 3 and another was positive for RSV-B. The patient who was in ICU was equivocally determined to have a rhinovirus infection. Results were repeated to definitively determine the diagnosis. All the other patients were negative for the viruses tested. These preliminary results have been communicated to Dr Jennifer of Kijabe Hospital. On subsequent days, diagnosis was done for ¿ human corona viruses including SARS, MERS CoV, etc ¿ human metapneumovirus ¿ Human herpes simplex type 1 ¿ Bocavirus All were negative. March 11 to March 22 The VHF entomology team went to Mombasa to conduct entomologic survey at the request of the Ministry of Health for the dengue fever outbreak. USAMRU-K GEIS team conducted: ¿

Sampling in areas neighbouring the KDF, Nyali. ¿ KDF Nyali has reported over ten cases since the beginning of the year. Preliminary results and general observations ¿ Over 1,000 Aedes Stegomyia mosquitoes were sampled with the possibility that the majority are Aedes aegypti. ¿ Both BG sentinel and CDC light traps were more successful at night as opposed to during the day. ¿ CDC light traps can effectively complement BG sentinels in trapping of Ae. aegypti mosquitoes. Indoor collections by Back pack aspiration were few and mainly Culex species. March 31 to April 12 GEIS annual QA/QC and Safety inspections was done in Kisumu, Kericho, inspections for the Nairobi laboratories will be conducted this week April 11 At the request of the KDF, USAMRU-K GEIS conducted training to over 20 KDF staff on the proper usage of PPE's , training was successfully conducted at the Military Memorial Hospital in Nairobi. March and April 2014. USAMRU-K GEIS was requested to assist with ID/AST laboratory testing for two Armed Forces memorial hospital (AFMH) patients Patient 1 was a burn patient in the ICU with persistent fever and infected wounds who was failing antimicrobial therapy. We performed identification and antimicrobial susceptibility testing of organisms cultured from the patient's wound swabs and blood cultures. Two organisms were identified as causes of wound infection: Acinetobacter baumannii and Pseudomonas aeruginosa. Drug susceptibility patterns indicated that although the Acinetobacter baumannii was multidrug-resistant, both organisms were sensitive to amikacin and tobramycin antibiotics. Blood cultures were negative. The patient was switched to aminoglycoside therapy and recovered. Patient 2 had a non-healing ulcerated lesion on the leg for 3 months with recurrent bacterial infections. We performed identification and antimicrobial susceptibility testing of organisms cultured from the patient's wound swabs. Two pathogenic organisms were isolated : Pseudomonas aeruginosa and a MDR vancomycin resistant Enterococcus casseliflavus sensitive to ciprofloxacin, Gent. Synergy and Strep. Synergy antibiotics. Results were transmitted but no information yet on patient outcome.

In Q3, the main activities this quarter were audits, protocol tracking for review and submissions of continuous Review Reports to the Institutional Review Boards (IRBs), trainings and review of the Standard Operating Procedures (SOPs). Standard Operating Procedures (SOPs) review development and distribution: The process of laboratory SOPs was ongoing during this reporting period. A total of 53 SOPs were reviewed, of these 38 were approved and the remaining are in the process of review processes. This mainly involves implementation of recommendations made by the reviewers. Quality safety and Regulatory Audits: The annual QAQC and Safety inspections were completed during this reporting period. The inspections were conducted in all the main laboratories and the satellite centers located in the Western region of the country. ISO 9001:2008 external audit was conducted at USAMRU-K Kericho as part of the requirement by Kenya Medical Research Institute. Corrective Action Plan for the non- conformities noted during the ISO audit was developed. All the non-conformities noted during the external audit were addressed and the missing documents developed and submitted to KEMRI Quality Management Systems office for review. The non –conformities noted during a previous internal audit (conducted during the previous reporting period) were also addressed. A closeout report will be issued by the KEMRI QMS team upon review of the implementation of the Corrective Actions. Regulatory Section: The department is responsible for review of new protocols, protocol submissions and ensuring compliance of the DEID protocols based in Nairobi. Four protocols submitted the Continuous Review Reports (CRRs) to KEMRI Ethical Review Committee and WRAIR Human Subject Protection Branch during this reporting period. Of these one has been granted approval for continuation for the next years by both Institutional Review Boards. One protocol amendment was submitted to the KEMRI Ethical Review Committee and WRAIR Human Subject Protection Branch (HSPB). There were several correspondences between the investigators and the WRAIR HSPB personnel. This was in regard to previously submitted protocols and assistance was provided in clarifying issues raised. Ensuring investigators' compliance with the guidelines was done through Providing assistance to investigators in providing responses, clarifications, and review

of protocols and submission of required documents. Protocol tracking was done and investigators advised on the appropriate action (example submission of CRRs or conduct human subject protection training). Conducting internal quarterly regulatory file audit. This was conducted for two Protocols based in Nairobi during this quarter. Two regulatory audits were conducted during this reporting period; The USAMRU-K regulatory affairs conducted an audit on one protocol based in Nairobi (WRAIR # 1267). A desk audit was conducted on one protocol affiliated to USAMRU-K through one of its investigators upon request by the WRAIR Institutional Review Board. 4. Archive routine maintenance and archiving: Monthly cleaning and maintenance of the archive. This involves housekeeping (dusting and cleaning of the surfaces, equipment and floor) and ensuring that the fire extinguishers were in good condition. 5. Trainings: The personnel in the department conduct mandatory safety and quality management training for all the personnel and interns allocated to work in the laboratory within the DEID program. There are 4 safety modules; Chemical hygiene & inventory, Fire safety & evacuation, Blood borne pathogens & Infection Control and a module on basic principles in quality systems and Good Laboratory Practices. During this reporting period; 14 interns from local universities and 6 personnel from the Kenya Defense Force personnel were trained. The director regulatory affairs in USAMRU-K conducted a two days training for investigators / research scientists based in Nairobi on WRAIR HSPB policies. A total of 12 research scientists / investigators attended the training. 6. Safety: Occupational Health: The department was in the process of providing Hepatitis B vaccinations for the personnel who were at risk within USAMRU-K in Nairobi. During this reporting period, one personnel completed the vaccination regime and three others received two of the three doses as stipulated. The three are scheduled to receive the final booster in December. Fire safety equipments maintenance: An inventory of all the fire equipments was taken awaiting the annual service during the next reporting period. A fire drill was conducted in Nairobi station and a report filed. Research oriented KDF medical officers were given material to read to be able to take the CITI training and be added to protocols From April to June 2014, the VHF laboratory at the request of the MoH processed a total of 69 clinical samples from 10 health facilities. The samples were tested for the presence of IgM antibodies for various arboviruses and viral hemorrhagic fever agents. 1 sample from Nairobi hospital tested positive for Dengue IgM antibodies. In addition, the laboratory also ruled out rift valley fever from 10 samples received from Muranga County who had asked specifically for rift valley fever testing on the samples. The results are summarized in the attached table. COL Rodney Coldren along with COL Mbinda (Senior Medical Officer Kenya Army) officially inaugurated the renovated laboratory at the Eldoret Military base.

In Q4, the fund is used to assist the Government of Kenya (GoK) in supporting the testing of the Ebola Virus Disease (EVD) Epidemic in West Africa and the Marburg outbreak in Uganda. The AFHSC-GEIS funded VHF laboratory has been designated the reference laboratory for the epidemic by the GoK, and in the past 3 months our laboratory has been operating 24 hours/7 days a week with 6 staff on standby to ensure that the laboratory was adequately staffed in the event we receive a call to analyze a suspect case at any time during the day or night. To date the VHF laboratory has tested 27 suspect samples with RT PCR and all were negative for Ebola and Marburg. USAMRU-K GEIS continues to be at the forefront of this emergency, furthermore, we are noticing an increase in the number of suspect cases in the past few weeks and have been warned by the MoH to be prepared for an sharp increase in suspect cases . (see MoH letter attached) Dr Rosemary Sang and Mr Victor Ofula from the VHF laboratory have been designated by the director of KEMRI to be his principal advisors on all Ebola/Marburg related activities On August 26, the KDF requested USAMRU-K DEID personnel to provide training on Ebola preparedness, (see KDF letter attached) The training was conducted at the KDF headquarters. Approximately 20 military personnel consisting of medical doctors, clinical officers, nurses, laboratory technologists and public health officers selected from different sites were present. The training covered the following topics: 1.

Introduction to Ebola Virus Disease (definition, mode of transmission, pathogenesis, symptoms, diagnosis and management), 2. Wearing and Removal of personal protective equipment (PPE) – this was done practically and theoretically 3. Sample collection, packaging and transportation, 4. Contact tracing, 5. Transportation of a patient with Ebola to an appropriate Health Facility, 6. Movement of an Ebola Patient within the Health Facility to Isolation ward, 7. Guidelines for the Clinical Management of Ebola 8. Waste management. SOP's were also provided to the participants and the KDF leadership for distribution. Trained individuals will train their staff at their respective location. Eventhough 1000 PPE's were requested USAMRU-K GEIS donated 200 PPE's to the KDF (see attached pictures) Outbreak investigation: A total of 73 samples were received under outbreak investigation. These samples were tested for arboviruses and VHFs. Of these 3 Samples tested positive for Rift Valley fever. This is the first time we are receiving RVF human cases outside of the EL-nino phenomena. Additionally 4 human samples tested positive for Dengue virus. Five samples were positive for Dengue Virus four of these samples were from the Coast while one came from the KDF

KEY RESEARCH ACCOMPLISHMENTS:

Supported the KEMRI Viral Hemorrhagic Fever Laboratory to test suspect samples for Ebola and Marburg Virus, among other VHFs, serving as the reference laboratory for the Kenyan MoH and the region and provided training to USAMRU-K, KEMRI, Ministry of Health, Kenya Defense Forces, and African Union Mission to Somalia (AMISOM) medical providers on the proper use of PPE and safe handing and shipping of suspect EBV samples

Training 301 clinicians and laboratory technicians, both military and civilian, on accurate malaria microscopy, further enhancing the Malaria Diagnostic Center's role a s the leading malaria diagnostic training center in East Africa

Establishing linkage between HDSS and hospital/clinic data to provide improved access to patient information to clinicians caring for this population and to enhance data capture for individuals enrolled in USAMRU-K studies (see vignette)

Determining the etiology and antimicrobial resistance patterns of enteric illnesses in Kenya, with 781 samples tested from across the country

Documenting the evolving resistance patterns of malaria in the various regions of Kenya and establishing molecular markers for resistance while preparing multiple manuscripts and abstracts to disseminate these findings (see vignette)

Training microscopists on accurate malaria diagnostic techniques and QA/QC procedures

Performing disease surveillance across Kenya, despite security and terrorism-related travel restrictions in the North, Northeast, and Coast

Training 107 junior laboratory students in quality, accurate laboratory diagnostics

Potentially finding the first case of documented MERS-CoV in Sub-Saharan Africa

REPORTABLE OUTCOMES: See references.

CONCLUSION:

KEMRI provides critical support to USAMRU-K's emerging infectious disease surveillance program in Kenya. Without KEMRI, USAMRU-K would not be able to execute its mission. KEMRI provides the legal and regulatory framework, personnel, and laboratory structure necessary to carry out scientific work. The organizations exist in partnership, with USAMRU-K working fully under the KEMRI umbrella in Kenya. Together, we have made great strides in establishing surveillance capabilities in the areas of respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

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